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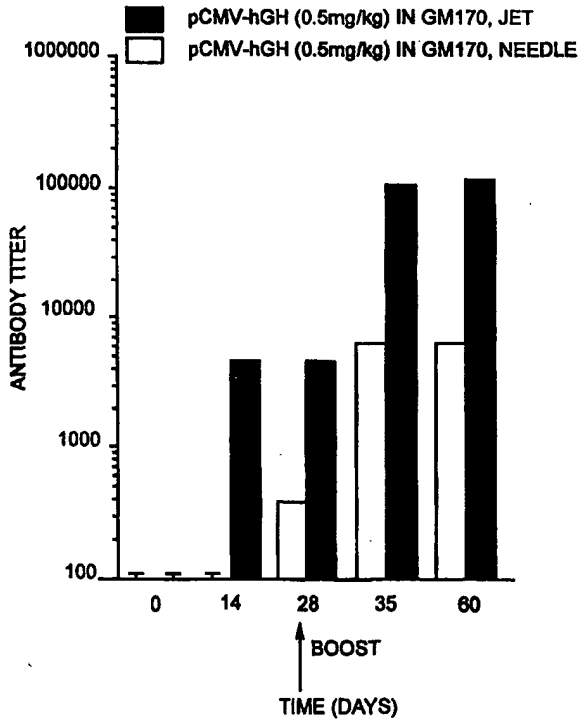
(54) **Title:** NEEDLE–FREE INJECTION OF FORMULATED NUCLEIC ACID MOLECULES

(57) **Abstract**

A novel method is provided for delivering nucleic acid molecules through and/or to the skin of mammals by needle–free injection. The method involves the incorporation of formulated nucleic acid molecules with devices for injecting the molecules by air, fluid and/or mechanical pressure. Disclosed are compositions and methods for enhancing the administration to and uptake of nucleic acids in a mammal. The methods disclosed provide an increased immune response by allowing the uptake of formulated nucleic acid molecules by a wide variety of cell types simultaneously. Also disclosed are examples which demonstrate that the combination of formulated nucleic acid molecules and needle–free injection methods results in immune responses which are superior to those obtained by conventional means of delivery. Methods for delivery, as well as methods for formulating nucleic acid molecules with various compounds, such as cationic complexing agents, polymeric and non–polymeric formulations, protective, interactive, non–condensing systems are also disclosed.



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TITLE: Needle-free injection of formulated nucleic acid molecules

INVENTOR: BARRY, M; MUMPER, R ; SMITH, L

PRIORITY-DATA: 1997US-069754P (December 16, 1997)

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ABSTRACTED-PUB-NO: WO 9931262A
BASIC-ABSTRACT:

NOVELTY - Delivery of nucleic acid molecules through and/or to the skin of mammals by a needle-free injection is new.

DETAILED DESCRIPTION - A method for delivering a nucleic acid molecule to a mammal comprises the step of providing the nucleic acid molecule formulated with a transfection facilitating agent through and/or to the skin of the mammal by use of a needle-free device configured and arranged to cause aerosol delivery of the nucleic acid molecule through and/or to the skin of the mammal.

INDEPENDENT CLAIMS are also included for:

(1) a kit comprising a provider for providing a nucleic acid molecule formulated with a transfection facilitating agent and a needle-free device for delivering the nucleic acid molecule through and/or to the skin of a mammal;

(2) a method for making a kit as above;

(3) a method of formulating nucleic acid molecules with modified inert particles; and

(4) a method of modifying gold particles by washing the gold particles in fuming or mixed acid solutions and combining the gold particles with cationic DNA binding peptides to form a monolayer coating.

USE - The method induces an immune response in a mammal, such as a human. The immune response may be humoral, T-cell mediated, prophylactic or therapeutic. The method and needle-free device can be used to treat a mammal suffering from a disorder conventionally treated by administering human growth hormone, for treating cancer, especially melanoma (and the nucleic acid encodes a cancer antigen, especially MAGE-1) or for treating an infectious disease, e.g. hepatitis B (the nucleic acid encodes a HBV core antigen). All claimed.

CLAIMS:

1. A method for delivering a nucleic acid molecule to a mammal comprising the step of providing said nucleic acid molecule formulated with a transfection facilitating agent through and/or to the skin of said mammal by use of a needle-free device configured and arranged to cause aerosol delivery of said nucleic acid molecule through and/or to the skin of said mammal.

2. The method of claim 1, wherein said nucleic acid molecule is DNA.

3. The method of claim 1, wherein said nucleic acid molecule is a plasmid with a eukaryotic promoter which expresses a therapeutic molecule.

4. The method of claim 3, wherein said therapeutic molecule is for human growth hormone.

5. The method of claim 1, wherein said nucleic acid molecule is RNA.

6. The method of claim 1, wherein said transfection facilitating agent is a protective, interactive and non-condensing compound.

7. The method of claim 1, wherein said transfection facilitating agent is selected from the group consisting of: one or more polyvinyl-pyrrolidones, one or more cationic lipids, one or more liposomes, one or more peptides, one or more modified gold particles, and one or more lipopeptides.

8. The method of claim 1, wherein said method results in an antibody response.

9. The method of claim 8, wherein said antibody response is at least 3 times greater than the antibody response caused by needle injection of the nucleic acid molecule suspended in saline.

10. The method of claim 9, wherein said antibody response is at least 10 times greater than the antibody response caused by needle injection of the nucleic acid molecule formulated with polyvinyl-pyrrolidone.

11. The method of claim 1, wherein said method induces an immune response.

12. The method of claim 11, wherein said immune response is a humoral immune response.

13. The method of claim 11, wherein said immune response is a T-cell mediated immune response.

14. The method of claim 11, wherein said immune response is a prophylactic immune response.

15 The method of claim 11, wherein said immune response is a therapeutic immune response.

16. The method of claim 1, wherein said mammal is a human.

17. The method of claim 1, wherein said needle-free means for delivering is a needle-free device that injects said nucleic acid molecule by aerosol delivery through and/or to the skin of a mammal.

18. The method of claim 1, wherein said needle-free means for delivering is a gas pressure device.

19. The method of claim 1, wherein said needle-free means for delivering is a mechanical spring device.

20. The method of claim 1, wherein said needle-free means for delivering is a multi-port device.

21. A kit comprising a provider for providing a nucleic acid molecule formulated with a transfection facilitating agent and a needle-free device for delivering said nucleic acid molecule through and/or to the skin of a mammal.

22. The kit of claim 21, wherein said nucleic acid molecule is DNA.

23. The kit of claim 21, wherein said nucleic acid molecule is a plasmid with a eukaryotic promoter which expresses a gene.

24. The kit of claim 23, wherein said gene is human growth hormone.

25. The kit of claim 21, wherein said nucleic acid molecule is RNA.

26. The kit of claim 21, wherein said tranfection facilitating agent is a protective, interactive and non-condensing compound.

27. The kit of claim 21, wherein said transfection facilitating agent is selected from the group consisting of: one or more polyvinyl-pyrrolidones, one or more cationic lipids, one or more liposomes, one or more modified gold particles, one or more peptides, and one or more lipopeptides.

28. The kit of claim 21, wherein said needle-free device for delivering is a needle-free device that injects said nucleic acid molecule by aerosol delivery through and/or to the skin of a mammal.

29. The kit of claim 21, wherein said needle-free device for delivering is a gas pressure device.

30. The kit of claim 21, wherein said needle-free device for delivering is a mechanical spring device.

31. The kit of claim 21, wherein said needle-free device for delivering is a multi-port device.

32. A method for making a kit comprising the steps of combining (*i*) a provider for providing a formulated nucleic acid molecule formulated with a transfection facilitating agent with (*ii*) a needle-free device for delivering said nucleic acid molecule through and/or to the skin of a mammal.

33. The method of claim 32, wherein said nucleic acid molecule is DNA.

34. The method of claim 32, wherein said nucleic acid molecule is a plasmid with a eukaryotic promoter which expresses a gene.

35. The method of claim 34, wherein said gene is for human growth hormone.

36. The method of claim 32, wherein said nucleic acid molecule is RNA.

37. The method of claim 32, wherein said tranfection facilitating agent is a protective, interactive and non-condensing compound.

38. The method of claim 32, wherein said transfection facilitating agent is selected from the group consisting of: one or more polyvinyl-pyrrolidones, one or more cationic lipids, one or more liposomes, one or more modified gold particles, one or more peptides, and one or more lipopeptides.

39. The method of claim 32, wherein said needle-free device for delivering is a needle-free device that injects said nucleic acid molecule by aerosol delivery through and/or to the skin of a mammal.

40. The method of claim 32, wherein said needle-free device for delivering is a gas pressure device.

41. The method of claim 32, wherein said needle-free device for delivering is a mechanical spring device.

42. The method of claim 32, wherein said needle-free device for delivering is a multi-port device.

43. A method of treating a mammal suffering from a disorder conventionally treated by administering human growth hormone, comprising the step of providing a nucleic acid molecule formulated with a transfection facilitating agent encoding human growth hormone through and/or to the skin of said mammal by use of a needle-free device configured and arranged to cause aerosol delivery of said nucleic acid molecule through and/or to the skin of said mammal.

44. The method of claim 43, wherein said mammal is a human.

45. The method of claim 43, wherein said tranfection facilitating agent is a protective, interactive and non-condensing compound.

46. The method of claim 43, wherein said transfection facilitating agent is selected from the group consisting of: one or more polyvinyl-pyrrolidones, one or more cationic lipids, one or more liposomes, one or more modified gold particles, one or more peptides, and one or more lipopeptides.

47. The method of claim 43, wherein said needle-free device for delivering is a needle-free device that injects said nucleic acid molecule by aerosol delivery through and/or to the skin of a mammal.

48. The method of claim 43, wherein said needle-free device for delivering is a gas pressure device.

49. The method of claim 43, wherein said needle-free device for delivering is a mechanical spring device.

50. The method of claim 43, wherein said needle-free device for delivering is a multi-port device.

51. A method of treating a mammal suffering from cancer, comprising the step of providing a nucleic acid molecule formulated with a transfection facilitating agent through and/or to the skin of said mammal by use of a needle-free device configured and arranged to cause aerosol delivery of said nucleic acid molecule through and/or to the skin of said mammal, wherein said molecule encodes a cancer antigen.

52. The method of claim 51, wherein said mammal is a human.

53. The method of claim 51, wherein said cancer antigen is MAGE 1, and said cancer is melanoma.

54. The method of claim 51, wherein said tranfection facilitating agent is a protective, interactive and non-condensing compound.

55. The method of claim 51, wherein said transfection facilitating agent is selected from the group consisting of: one or more polyvinyl-pyrrolidones, one or more cationic lipids, one or more liposomes, one or more modified gold particles, one or more peptides, and one or more lipopeptides.

56. The method of claim 51, wherein said needle-free device for delivering is a needle-free device that injects said nucleic acid molecule by aerosol delivery through and/or to the skin of a mammal.

57. The method of claim 51, wherein said needle-free device for delivering is a gas pressure device.

58. The method of claim 51, wherein said needle-free device for delivering is a mechanical spring device.

59. The method of claim 51, wherein said needle-free device for delivering is a multi-port device.

60. A method of treating a mammal suffering from an infectious disease, comprising the step of providing a nucleic acid molecule formulated with a transfection facilitating agent through and/or to the skin of said mammal by use of a needle-free device configured and arranged to cause aerosol delivery of said nucleic acid molecule through and/or to the skin of said mammal, wherein said molecule encodes an antigen for said infectious disease.

61. The method of claim 60, wherein said mammal is a human.

62. The method of claim 60, wherein said infectious disease antigen is HBV core antigen, and said infectious disease is chronic hepatitis.

63. The method of claim 60, wherein said tranfection facilitating agent is a protective, interactive and non-condensing compound.

64. The method of claim 60. wherein said transfection facilitating agent is selected from the group consisting of: one or more polyvinyl-pyrrolidones, one or more cationic lipids, one or more liposomes, one or more modified gold particles, one or more peptides, and one or more lipopeptides.

65. The method of claim 60, wherein said needle-free device for delivering is a needle-free device that injects said nucleic acid molecule by aerosol delivery through and/or to the skin of a mammal.

66. The method of claim 60, wherein said needle-free device for delivering is a gas pressure device.

67. The method of claim 60, wherein said needle-free device for delivering is a mechanical spring device.

68. The method of claim 60, wherein said needle-free device for delivering is a multi-port device.

69. A method of formulating nucleic acid molecules with modified inert particles comprising the steps of combining said nucleic acid molecules with said modified inert particles in an aqueous environment.

70. The method of claim 69, wherein said modified inert particles are modified gold particles.

71. A method of modifying gold particles comprising the steps of washing said gold particles in fuming or mixed acid solutions, and combining said gold particles with cationic DNA binding peptides to form a monolayer coating.

72. The method of claim 71, wherein said cationic DNA binding peptides have cysteine as the terminal residue.

73. The method of claim 71, wherein said fuming acid is nitric acid.

74. The method claim 71, wherein said mixed acid comprises nitric acid and sulfuric acid.

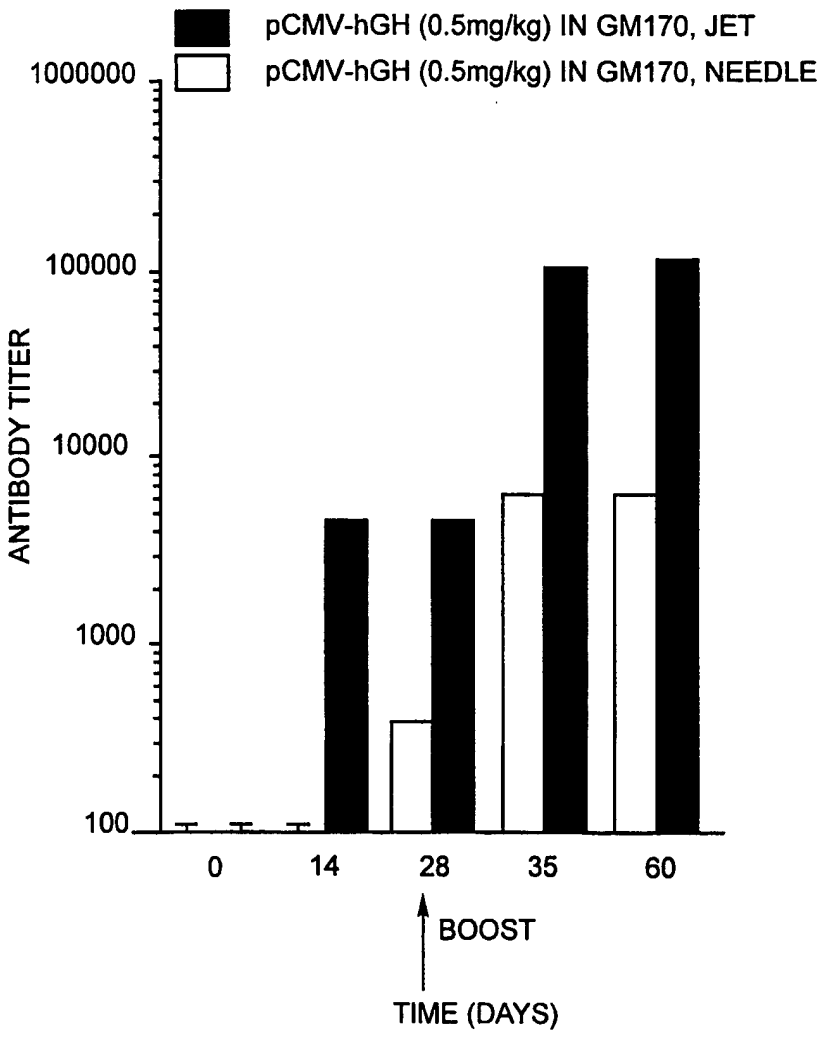
75. A method for delivering a nucleic acid molecule to a mammal comprising the step of providing said nucleic acid molecule formulated with modified gold particles through and/or to the skin of said mammal by use of a needle-free device configured and arranged to cause aerosol delivery through and/or to the skin of said mammal.

76. The method of claim 74, wherein said needle-free device for delivering is a needle-free device that injects said nucleic acid molecule by aerosol delivery through and/or to the skin of a mammal.

77. The method of claim 74, wherein said needle-free device for delivering is a gas pressure device.

78. The method of claim 74, wherein said needle-free device for delivering is a mechanical spring device.

79. The method of claim 74, wherein said needle-free device for delivering is a multi-port device.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pCMV-hGH (0.5mg/kg) IN GM170, NEEDLE
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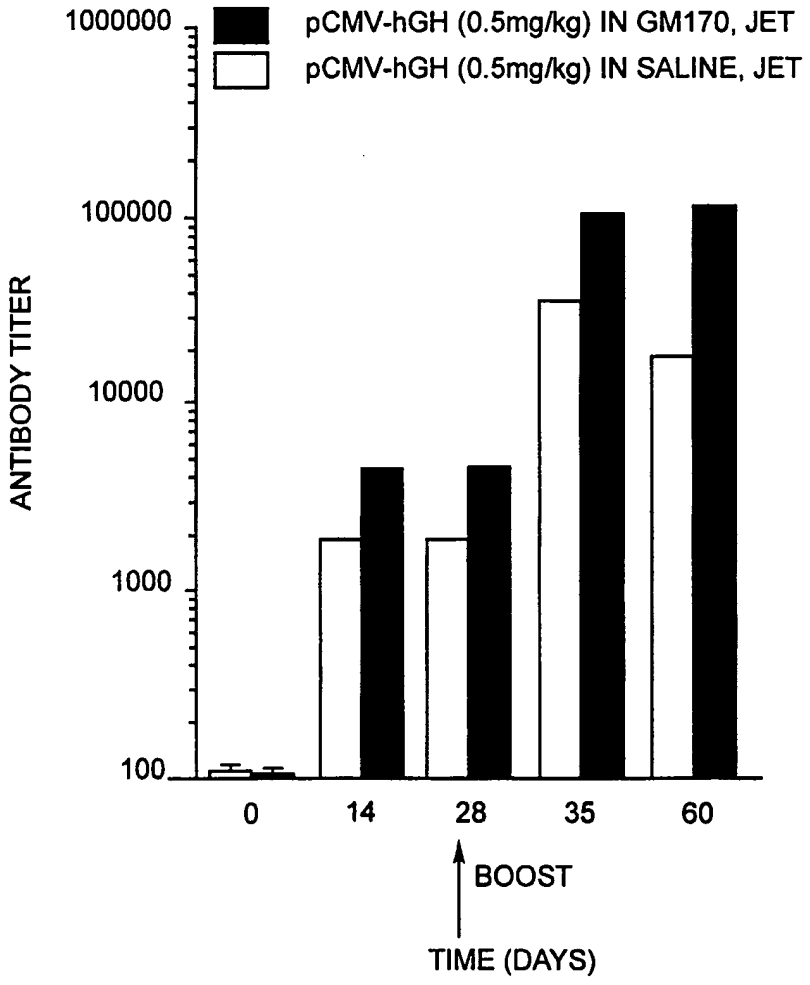
ANTIBODY TITER
0
14
28
35
60
BOOST
TIME (DAYS)

Fig. 1


1000000
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ANTIBODY TITER
pCMV-hGH (0.5mg/kg) IN GM170, JET
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0
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28
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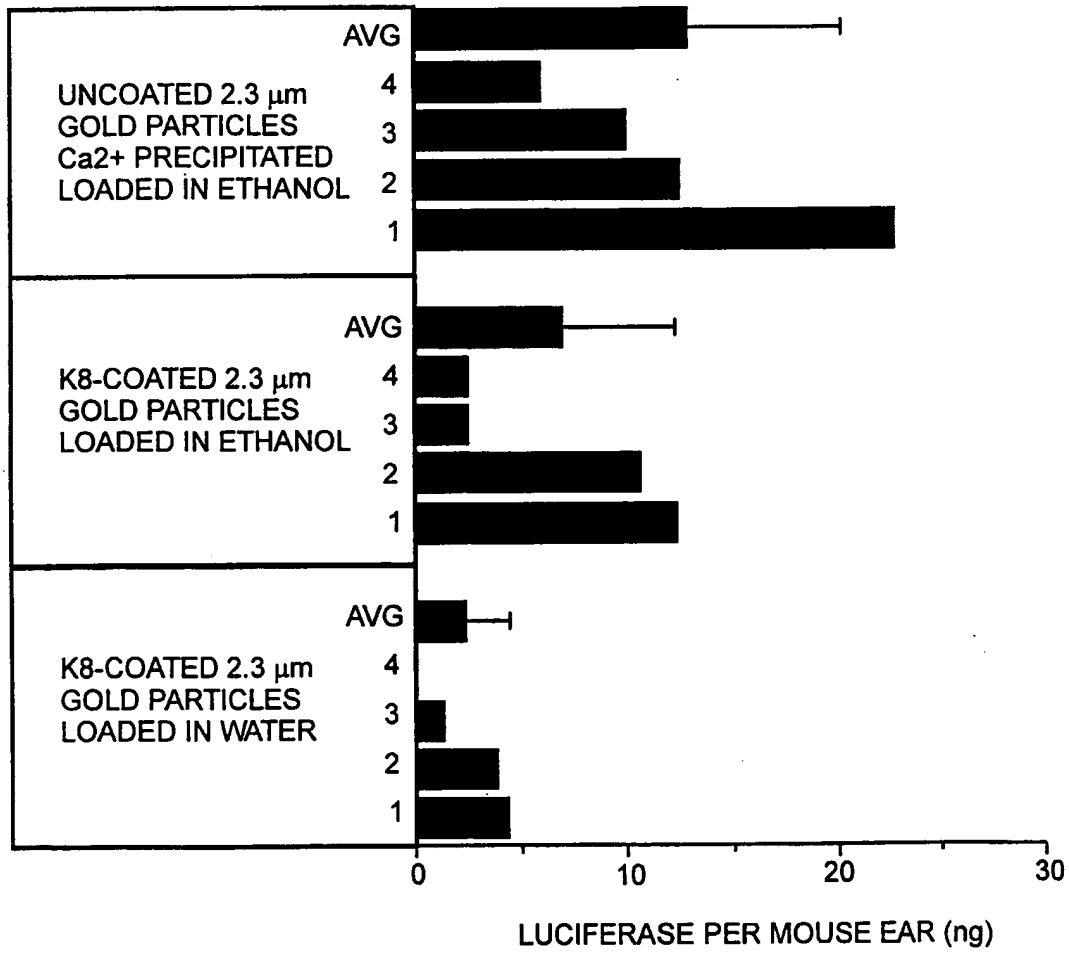
BOOST
TIME (DAYS)

Fig. 2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3
2
1
UNCOATED 2.3 µm
GOLD PARTICLES
Ca2+ PRECIPITATED
LOADED IN ETHANOL
AVG
4
3
2
1
K8-COATED 2.3 µm
GOLD PARTICLES
LOADED IN ETHANOL
AVG
4
3
2
1
K8-COATED 2.3 µm
GOLD PARTICLES
LOADED IN WATER
0
10
20
30
LUCIFERASE PER MOUSE EAR (ng)

Fig. 3